Can the Neuropathology of ALS Lead Towards a Definitive Biomarker?

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ABSTRACT

Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease, without a cure, that leads to progressive loss in muscle coordination, promoting muscular atrophy and bulbar palsy (McDermott and Shaw, 2008). ALS ultimately leads to respiratory failure, accompanied with pulmonary dysregulation, causing impairment of oculomotor nerves, thus paralyzing voluntary muscle movement and is thus fatal. As disease progression is rapid it can be challenging to treat as there is little time for the damage to motor neurons to be reversed, considering the increased muscle wastage.

The objective of this study is to understand the pathology of ALS to lead towards a possible biomarker that could diagnose ALS in pre-symptomatic stages. Biomarkers are molecules found in bodily fluids or tissue that can reveal biological characteristics. This enables clinicians to determinate if an individual is at risk of developing a specific disease or possible reaction to treatment (Lundbald, 2016). This would allow early detection and enable researchers to target those pathways and identify a possible treatment.

The major findings from this study indicate that familial ALS is due to either a gain or loss of protein function, arising from mutations and thus leading to neurotoxicity. In other cases, occurrence is random but environmental factors, or mitochondrial dysfunction may increase the probability of disease onset. Currently $p75^{ECD}$ (Shepheard et al., 2017) and neurofilament proteins (Rosengren et al., 2002) are the biomarkers used to distinguish ALS, however, they are not effective during pre-symptomatic stages. Although, recent advances in research are leading to the discovery of new biomarkers, neurological conditions have the ability to mimic each other making it challenging to identify a distinctive biomarker. Novel imaging techniques have also been developed to identify changes in the brain tissue providing a possible diagnosis to diseases like ALS.

A combination of biomarkers consisting of a biological compound and imaging tool would yield successful pre-symptomatic assessment and accurate reflection of disease progression for ALS.

INTRODUCTION

The brain is composed of elementary computational units known as neurons, which intercept membrane depolarization in the form of action potentials and transmit these electrochemical messages via synaptic communication. They coordinate the transmission of these signals to various parts of the brain and spinal cord, forming the central nervous system which further innervate and receives signals from the distant part of the body, via the peripheral nervous system. These neurons have a life cycle that includes birth, migration, differentiation, and death (www.ninds.nih.gov, 2022). Neurodegenerative disorders are a class of diseases characterized by the gradual loss of neurons in the central nervous system. The phenotype of neurons that are lost is connected with the beginning of distinct neurological disorders in various neurodegenerative diseases (Dailah, 2022). For example, Parkinson's Disease is recognized by neuronal loss in the substantia nigra, a region of the midbrain, whereas Huntington's Disease is distinguished by neuron loss in the striatum, a region of the brain's basal ganglia.

Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease that is distinguished by the gradual degradation of upper and lower motor neurons in the brain and spinal cord, as shown in Figure 1. ALS is a rare, incurable disease that affects approximately two people out of every 100,000 in the general population (Miller, Gelinas, and O'Connor, 2004). Disease onset usually occurs around the age of 65 years, however younger people can also be affected. (Assoni, Foijer and Zatz, 2022). ALS, represents both the degeneration of corticospinal motor neurons and the scarring of their axons in the lateral spinal cord, known as 'lateral sclerosis,' and motor neurons in secondary denervation and muscle wasting, known as 'amyotrophic,' as reported by neurologist Jean-Martin Charcot (Taylor, Brown, and Cleveland, 2016).

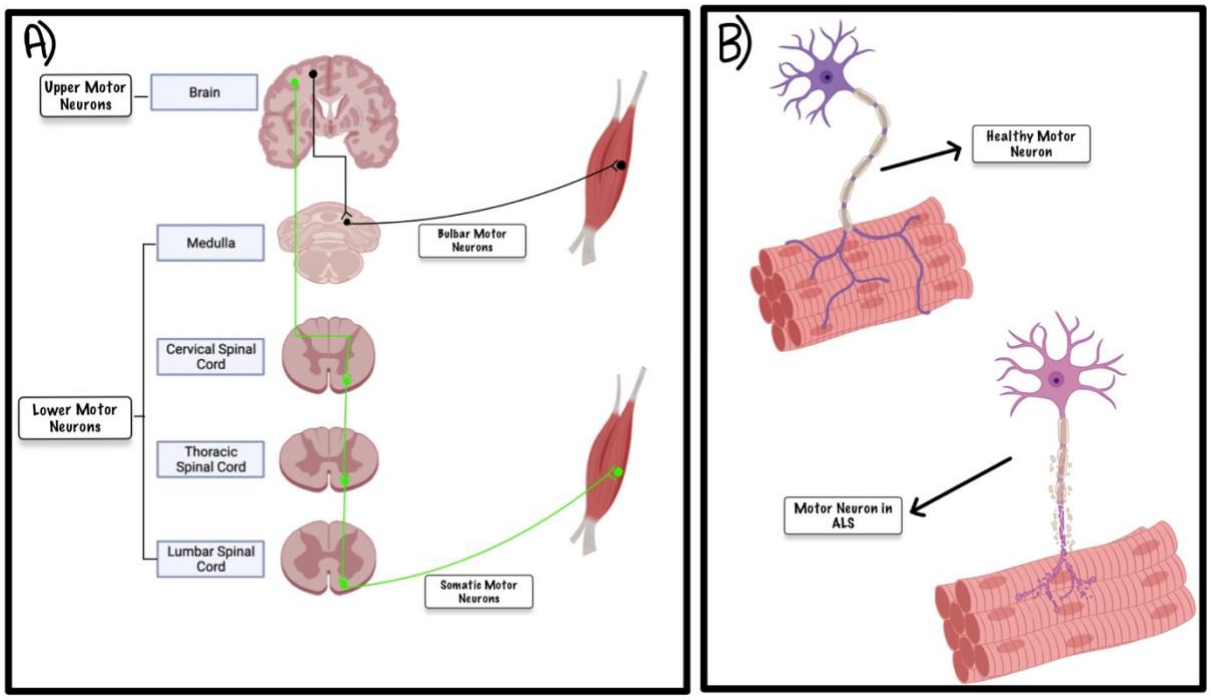

Figure 1: Motor Neurons Affected by ALS. A) Schematic representation of the pathway used to initiate muscle contraction. The bulbar motor neurons are also known as the lower motor neurons which initiate synchronized swallowing and breathing, progressive bulbar palsy degenerates these neurons in the brain stem region dysregulating muscle coordination and is one of the most prominent symptoms diagnosed in ALS. The upper motor neurons innervate the pyramidal tract and are present in the corticospinal region. They collectively innervate the musculature and other glands of the body. B) Comparison of a healthy motor neuron to motor neurons in patients with ALS. This illustration shows earlier signs of degeneration of the myelinated axons and axonal terminal degeneration which affects the ability of the cell to carry out its tasks in muscle contraction.

ALS affects the functioning of cortical and spinal motor neurons leading to a progressive loss in muscle coordination, promoting muscular atrophy and progressive bulbar palsy. Risk of disease development in males compared to females is higher since the ratio is between 1 and 3 (Manjaly et al., 2010), thus men are more susceptible to the disease. The epidemiology of the disease suggests that steady degeneration of the upper and lower motor neurons causes muscle atrophy in the advanced stages of the disease including damage to the brain stem (McDermott and Shaw, 2008). Localized or distal muscular atrophy is accompanied by progressive bulbar palsy and spasticity of respiratory muscles which leading to a distinct abnormal gait. Due to pseudo bulbar dystrophy, respiratory weakness progresses, and this contributes to nocturnal hyperventilation, anorexia, lack of concentration and disturbance in the sleep wake cycle. Speech disturbances develop within the primary years leading to sialorrhea and often cause facial paralysis. Advanced stage patients develop supranuclear gaze palsy which causes lack of posture control leading to cognitive loss. The cause of death in this disease is respiratory failure accompanied with pulmonary dysregulation which causes

impairment of oculomotor nerves, thus paralyzing voluntary muscle movement. Symptoms associated with bulbar lower motor neurons are usually identified as tongue weakening, palatal weakness and a nasal tone in the speech (Wijesekera and Leigh, 2009). In later stages of ALS bladder control becomes difficult, accompanied by dysregulated micturition and retardation of limb muscles leads to impaired mobility. Dysphagia develops due to lack of swallowing and respiratory malfunctioning that leads to pneumonia. Secondary symptoms include acute pain due to musculoskeletal degeneration and reduced muscle tone, excessive collateral axonal branching, uncoordinated movement of ligaments tendons and joints leading to cramps. There is very little time for the damage to be reversed considering the increased muscle wasting and weakness occurred during the disease. In this research project, the aim is to determine early occurrences that might contribute to the pathogenesis of ALS. An accurate pre-clinical diagnosing biomarker(s) could be identified that targets pathogenic pathways which leads to ALS phenotype. Therefore, it is crucial to gain a better understanding of the causes and pathophysiology of ALS.

ALS can be classed as sporadic or genetic. Sporadic cases occur without a familial history of the disease whilst genetic cases are caused by known gene mutations. According to Taylor, Brown, and Cleveland (2016), 10% of ALS cases are passed down through families, with dominant characteristics having a high penetrance. This means that genetic cases of ALS will almost always be apparent in the individual that carries the allele because of the high probability of the gene being expressed. With the evolution of molecular genetic techniques, extensive datasets generated from large scale DNA sequencing and first-generation methods indicate that there are several genetic factors and multiple risk of inheritance of genes that initiate the disease. Mostly autosomal dominant familial forms of ALS are same mutations in the SOD-1 gene are the most predominant cause in 20% of ALS patients characterized by the A4V mutation which leads to lower motor neuron disease (Taylor, Brown, and Cleveland, 2016). Other identifiable targets are TDP43 which regulates mRNA processing, cleavage, and mRNA stability.

1. FAMILIAL ALS

1.1 Superoxide Dismutase-1

The metalloenzyme superoxide dismutase-1 (SOD1) is found in eukaryotes and some prokaryotes. As shown in Figure 2, SOD is the first line of defence against O^{2-} toxicity because catalysing the dismutation of two molecules of O^{2-} to hydrogen H_2O_2 and O_2 reduces O^{2-} availability, thus reducing the number of free radicals (Wang et al., 2018). The two prominent mutations involved are point mutations of glycine 85 to arginine (G85R) and glycine 93 to alanine (G93A) leading to neurotoxic functions of SOD-1 disease progression (Kunst et al., 1997). The first ALS-causing mutation was discovered in the superoxide dismutase 1 (SOD1) gene in 1993 (Miller, Gelinas, and O'Connor, 2004), and since then, 15% of hereditary forms of ALS have been related to one of over 100 distinct SOD1 mutations (Strong, Kesavapany and Pant, 2005).

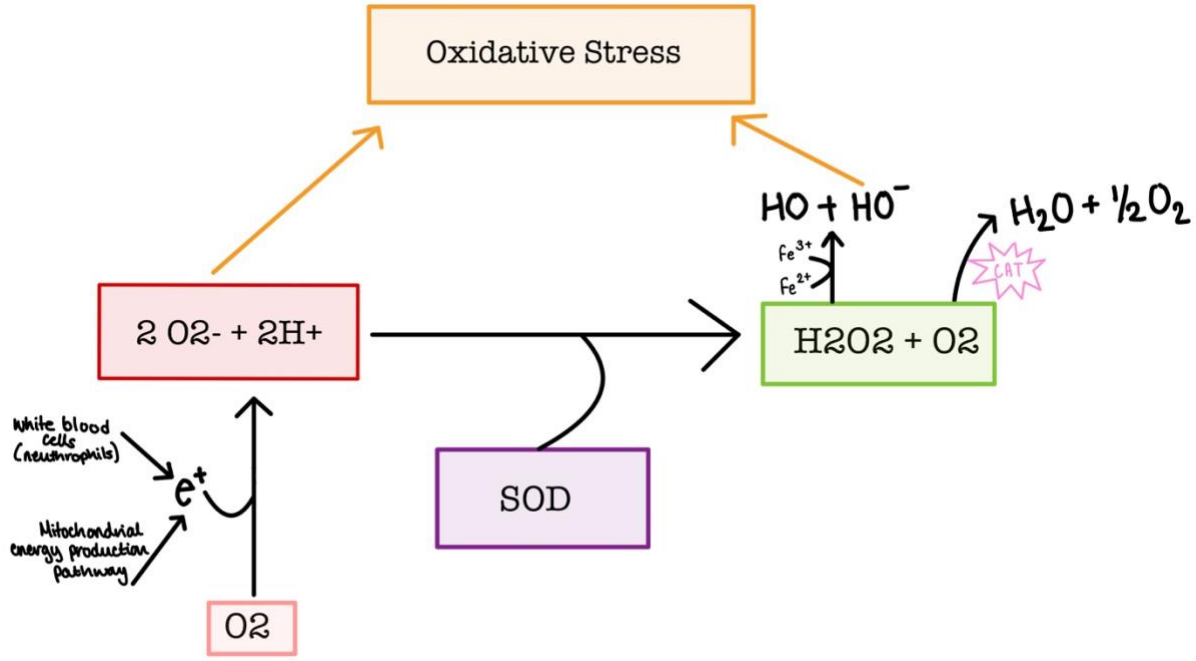

Figure 2: Superoxide Dismutase (SOD) catalysis of O^{2-}. The conversion of the superoxide anion free radical, O^{2-}, to hydrogen peroxide, H_2O_2, and oxygen (O_2) is catalysed by SOD. The catalase enzyme (CAT) can reduce H_2O_2 to water. In the presence of Fe2+, H2O2 can create another reactive oxygen species (ROS) known as hydroxide ion (OH). These free radicals contribute to the damage caused by oxidative stress.

These mutations induced in familial ALS led to protein misfolding as there are no primary hotspot mutations, and the entire structural conformation of the protein changes contributing to ALS phenotype, neuronal transport alterations and mitochondrial as well as proteasome

aggregation defects. This increases the likelihood of accumulation and prion-like conversion. Furthermore, SOD1 amyloid fibrillation is increased to variable degrees by ALS-associated mutations. SOD1 mutations that cause ALS impair folding stability and allow the protein to attain more aggregation-prone conformations (Strong, Kesavapany and Pant, 2005).

There is no indication that mutant SOD1 toxicity is explained by either decreased or enhanced SOD1 activity (Khare et al., 2005). The elevated toxicity could be caused by the loss of function SOD1 which induces oxidative damage causing intracellular misfolding or aggregation. Both transgenic mice producing mutant SOD1 protein and familial ALS patients with a SOD1 mutation have ubiquitin-immunoreactive intraneuronal and astrocytic mutant SOD1 protein aggregates. SOD1 aggregates may cause neurotoxicity by interfering with normal proteasomal function or by changing chaperone interactions (Strong, Kesavapany and Pant, 2005). Recently, a direct connection between mutant SOD1 and mitochondria was also discovered (Tan et al., 2014). Apoptosis and neural mitochondrial dysfunction are both important metabolic events in the aetiology of ALS.

1.2 Transactive Response DNA-Binding Protein 43

TAR DNA-binding protein 43 (TDP-43) is highly involved in RNA processing. It is identified as a pathological protein in ALS and regulating frontal temporal degeneration caused due to ubiquitination. Hyperphosphorylated fragments of this protein accumulates in neural cells leading to neurodegenerative effect and cognitive deficiency (Prasad et al., 2019). Missense mutations can result in the production of a different amino acid due to change in a single nucleotide of the expressing gene. TDP-43 proteinopathies indicate a rare missense mutation in the gene encoding TDP-43 (TARDBP) which may cause ALS (Mackenzie and Rademakers, 2008). Many studies indicate that TDP-43 and its immunoreactivity contribute to poor prognosis in ALS –Parkinson-Dementia complex leading to cortico-basal degeneration (Boeve, 2007).

Messenger RNA (mRNA) stability, mRNA processing, mRNA translation and transport, and negative regulation of alternative splicing are only a few of the cellular functions carried out by the heterogeneous nuclear ribonucleoprotein. The majority of ALS cases have been documented to have nuclear TDP-43 depletion and the development of pathogenic aggregates

in the cytoplasm to further increase toxicity (Prasad et al., 2019). Although the exact mechanism causing this redistribution is unknown, it is likely either a faulty TDP-43 cytoplasm to nucleus transport mechanism or TDP-43 translocation from nuclei to cytoplasm. According to Saberi et al. (2015), immunoblot analysis discovered a 45kDa band that represented a phosphorylated TDP-43. This finding suggested that TDP-43 may have undergone a post-translational modification, which might be involved in redistribution.

Approximately 30 mutations have been found in the TARDBP gene, which encodes TDP-43, with the majority of them located in the glycine-rich region, that may be responsible for regulating the DNA binding potential and protein-protein interactions (Saberi et al., 2015). Analysing the protein's specialised single domain has assisted scientists better grasp the biophysical qualities of TDP-43. The low complexity domain (LCD) contains the vast majority of ALS-related mutations. The TDP-43 low-complexity domain is referred to as the prion-like domain due to the high concentration of asparagine, glutamine, glycine, and tyrosine residues present to form the amyloid core complex (Prasad et al., 2019). Liquid-liquid phase separation (LLPS) is a mechanism which explains the formation of organelles or membrane-less molecules to stabilise the order in cells. Due to LLPS in the LCD, wild type TDP-43 can produce oligomeric structures that increase toxicity. The independent interactions between TDP-43 molecules were regulated by the N-terminal domain (NTD), which may prevent LCD-mediated TDP-43 aggregation. Suggesting that the NTD may have a protective function in ALS (Prasad et al., 2019). As a defence mechanism, the RNA binding properties have been connected to TDP-43 toxicity. TDP-43 mislocalization may prevent healthy RNA trafficking to the cytoplasm and encourage a milieu where TDP-43 is less soluble (Suk and Rousseaux, 2020).

1.3 Fused in Sarcoma

Fused in sarcoma (FUS) is a DNA- and RNA-binding protein that mostly resides in the nucleus of neurons which moves between the nucleus and cytoplasm. FUS is involved in DNA repair, regulation of transcription and RNA splicing and formation of dynamic ribonucleoprotein granules. Ribonucleoproteins aid RNA metabolism and are linked to memory and development. Missense mutations that accumulate in the cytoplasm are able to form stable ribonucleoprotein granules that can lead to inclusion bodies and contribute to neurotoxicity (Kamelgarn et al., 2018).

In 2009, Vance et al., identified a missense mutation in the gene encoding FUS that links to ALS. They found a base pair change in exon 15 of FUS which caused a substitution of arginine to cysteine at position 521 in the C-terminus of the protein. The C-terminus is involved in protein and RNA binding, while the N-terminus is important in transcriptional activation (Assoni, Foijer and Zatz, 2022). An additional base-pair change was found that resulted in a change from arginine to histidine at the 521 positions. The third mutation was found in exon 14 that resulted in a change from arginine to glycine substitution at position 514.

According to Svetoni, Frisone and Paronetto 2016, mutations in FUS have been demonstrated to result in aberrant splicing which could be explored further to understand ALS pathology. Frameshift and pretermination in exon 15 caused shortened FUS to lose its nuclear localization signal domain, increasing the mislocalization of FUS into the cytoplasm (Yang et al., 2022). These mutations cause LLPS of FUS protein to include cytoplasmic inclusions that interfere with RNA metabolism pathway (Kamelgarn et al., 2018) which can cause ALS-like symptoms.

1.4 C90RF72

C90RF72 is a gene found within locus 9p21 on chromosome 9 that, according to Renton et al. 2011, possesses an unexplained autosomal-dominant trait that contributes to ALS pathology. A protein, encoded by C90RF72, is essential for regulation of endosomal trafficking and forming interactions for endocytosis (www.ncbi.nlm.nih.gov, 2023). A GGGGCC hexanucleotide repeat expansion located 63 base pairs centromeric to the first exon of the C90RF72 transcript was found inside this region of the gene (Orr, 2011). In contrast to mutant alleles, which often have more than 30 repetitions, wild-type C90Rf72 alleles normally have 23 repetitions. The relationship between recurrent expansion in C90RF72 and neurodegeneration in ALS is currently unknown.

A common variant of ALS is frontotemporal dementia, which occurs due gradual damage to the temporal and frontal lobes of the brain. Those with both frontotemporal dementia and ALS (FTD-ALS) including a strong family history of the disease would have the greatest rate of C90RF72 expansion (Dobson-Stone et al. (2012)). In a subgroup of this cohort with a confirmed family history of ALS or early-onset dementia the frequency was 28.6%, but in the

presence of ALS in family history and C90RF72 expression, the frequency rose to 29.4% (Dobson-Stone et al., 2012). This increase means mutation is very likely to be carried by families with an autosomal dominant pattern of inheritance and by individuals who have both FTD and ALS.

There are two distinct types of ubiquitinated inclusions of C90RF72, one of them is similar to TDP-43 protein pathology. Mori et al 2013, discovered inclusions of dipeptide-repeat (DPR) proteins that formed by translation initiated from the expanded GGGGCC repeats. It has been widely researched that increased expression of mutant C90RF72 increases neurotoxicity due to aberrant RNA splicing (DeJesus-Hernandez et al., 2011, Renton et al., 2011). An increase in the transcripts containing intron 1, where repeat expansion is located, produced unnatural DPR through selective stabilisation. Since DPRs accumulate in the brain during early stages of disease onset, it would be beneficial to evaluate their deposition pattern in motor cortex and spinal cord to improve understanding of disease pathology. However, analysis through traditional methods of immunohistochemistry is difficult due to the charges and soluble nature of the DPRs (Freibaum and Taylor, 2017).

2. SPORADIC ALS

2.1 Environmental Exposures

Patients without family history of ALS are classified as having sporadic ALS which affects 90% of all cases (Talbott, Malek and Lacomis, 2016). Although environmental factors contribute to 40% of ALS cases (Al-Chalabi and Hardiman, 2013), current research on neurodegenerative illnesses solely focuses on two major areas: identifying and minimising risk factors for disease onset and discovering contemporary treatments to be put into practise. Therefore, the influence of environmental influences is not well understood. Given that certain neurons would be vulnerable to certain toxins, a small number of studies suggest that environmental exposures may affect the phenotype of ALS (Cannon and Greenamyre, 2011).

Since prominent athlete Lou Gehrig was diagnosed with ALS, media attention has been drawn to other renowned athletes who encountered similar diseases (Al-Chalabi and Hardiman, 2013). This increased curiosity of a possible link between sports and ALS, however, there are conflicting findings regarding the role of physical exercise and ALS. Some epidemiological studies indicate a correlation between head, neck, and back injury to strongly associate with risk of ALS (Strickland et al., 1996), that could be encountered during intense physical activity like professional sports. However, a recent study conducted by Gotkine, Friedlander and Hochner, 2014 found that primary bulbar onset in patients is related to vigorous exercise regardless of head and neck trauma. Since the later study contradicts Strickland et al 1996, trauma through injury might not be a key factor and perhaps exercise could be a risk factor into ALS onset. On the other hand, some studies hypothesize that physical activity may play a protective role in patients with ALS since clinical trial conducted by Drory et al., 2001 found improvements in motor function of ALS patients who performed daily stretching and resistance exercise. There was a decrease in loss of motor function, fatigue, and pain after 6 months of moderate daily exercise regimen. Due to conflicts in findings, it might be beneficial to address metabolic or genetic factors that could provide an alternative explanation.

The human immunodeficiency virus (HIV) has caused some patients to exhibit motor neuron disease symptoms (Rowland and Shneider, 2001). Nevertheless, this does not demonstrate that infections can result in ALS, and in some instances, anti-HIV therapy led to restore the motor neuron condition (Rowland and Shneider, 2001). Celeste and Miller (2018) found that

enteroviruses, retroviruses, and herpesviruses all possess neurotropism, which enables them to target and destroy neural tissue. As demonstrated in Figure 3, viruses can cause oxidative stress, protein misfolding, mitochondrial damage, and other ALS symptoms by targeting pathways which contribute to pathophysiological features of the condition. Individuals with an underlying genetic susceptibility for ALS may have an increased chance of developing the disease due to a virus-mediated disruption of pathways.

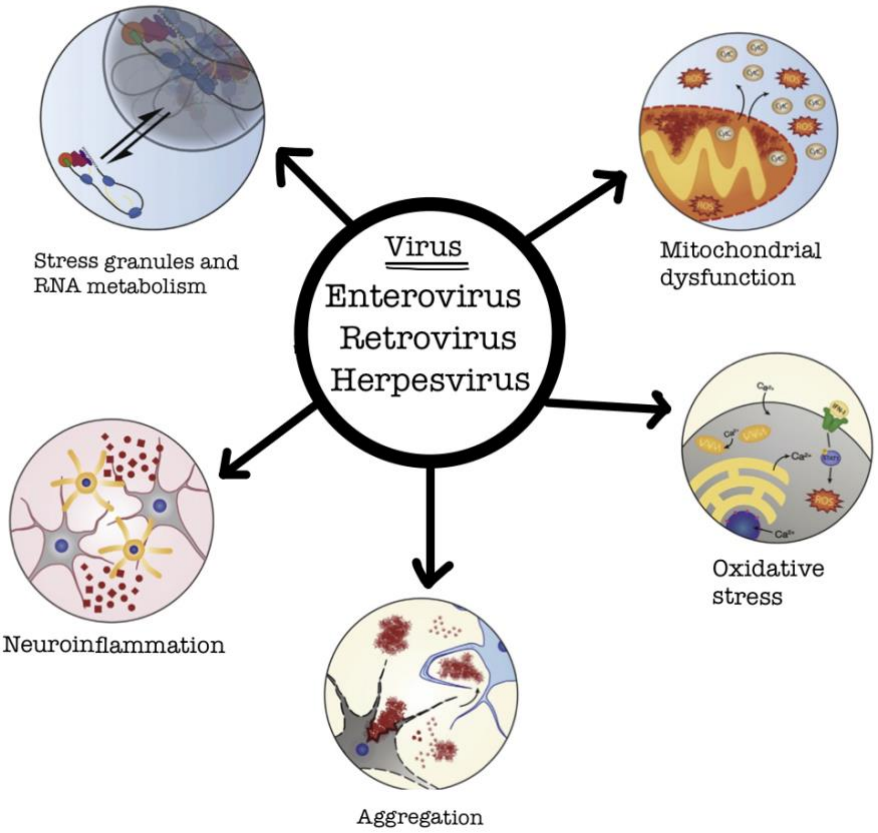

Figure 3: ALS cellular pathways affected during viral infection. Viruses, such as Enterovirus, Retrovirus or Herpesvirus survive in the host by manipulating the cellular pathways that are associated with ALS pathophysiology such as oxidative stress, inflammation, toxic protein aggregation, stress granule dynamics along with RNA metabolism and mitochondrial dysfunction. Certain pathways that are already under stress due to underlying genetic factors can trigger the onset of ALS or even speed up the disease progression upon viral attack. (Adapted from Celeste and Miller, 2018)

2.2 Smoking and alcohol consumption

It is universally accepted that smoking and alcohol consumption is injurious to health. Many studies have pursued to identify a link between smoking or alcohol consumption with ALS. Despite the fact that alcohol consumption did not increase the risk of ALS, current cigarette smokers have a three times higher likelihood of developing ALS than control (Nelson et al.,

2000). In addition, a multivariate analysis by Sutedja et al., 2007 identified an increased risk of developing ALS among cigarette smokers. These findings seem consistent with hypotheses that environmental toxins are risk factors for ASL, however a more recent case-control study contradicts these results. Pamphlett and Ward 2012 found no significant difference amongst ALS smoking patients and control group thus concluding that the study did not support a link between ALS and cigarette smoking. Perhaps rate of disease progression increases due to underlying cell damage caused by toxicants found in tobacco, which could cause early onset of later symptoms related to respiratory failure in ALS. It would be beneficial to conduct further research to determine how rate of disease progression is affected by toxins in cigarettes.

2.3 Oxidative Stress & Mitochondrial Dysfunction

Oxidative stress is a pathological aspect of ALS since excess of reactive oxygen species (ROS) and inadequate antioxidant defence can lead to mitochondrial malfunction resulting the neurodegeneration (Guo et al., 2013). However, under typical circumstances, the production and clearance of ROS are balanced. Inflammation may arise from DNA damage or cell tissue breakdown brought on by oxidative stress. Oxidative stress can increase when an organism is unable to maintain the proper ratio of ROS, or when its antioxidant defences are compromised (Cunha-Oliveira et al., 2020).

ROS comprise of superoxide radical anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (HO) and nitric oxide (NO). NO is produced by immune cells through nitric oxide synthase (NOS). 0_2^- react rapidly with NO to produce peroxynitrite ($ONOO^-$) which is a highly reactive oxidizing agent that have the ability to damage organic compounds such as proteins or lipids (Beckman and Koppenol, 1996). Misfolded proteins and modification in cellular membrane functions through oxidation of unsaturated fatty acids can arise from increased oxidization. Shibata et al., 2001 detected markers positive for lipid oxidation in spinal cords of patients who were diagnosed with sporadic ALS. Elevated NOS were observed in ALS motor neurons and increased immunoreactivity suggests reactive nitrogen species might contribute to ALS pathogenesis (Abe et al., 1997).

ROS are directed towards the mitochondrial matrix and complex III, as shown in Figure 4, leaks electrons to oxygen molecules on both sides of the inner mitochondrial matrix, O_2^- is

created at various locations in the mitochondria. Approximately 90% of intracellular ROS is produced by the mitochondria because 0.2 – 2% of molecular oxygen consumed is reduced to O_2^- and further converted to ROS (Tirichen et al., 2021). Cannon and Greenamyre (2011) state that Complex III is the primary location of radical production because free radicals assault these complexes and prolonged exposure can cause oxidative damage to proteins in the mitochondria and other cell structures.

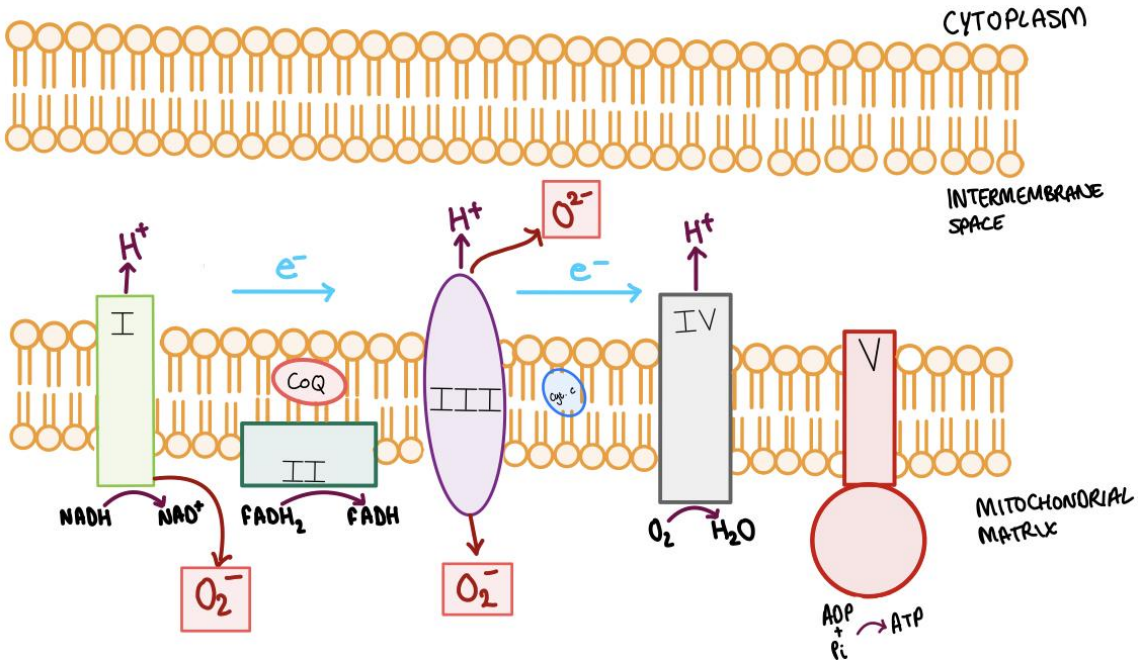

Figure 4: Mitochondrial respiratory chain. Hydrogen from organic acids such as pyruvate is oxidized in the electron transport chain. The respiratory complex I, shown in light green rectangle, is the NADH oxidoreductase which transfers electrons to the coenzyme Q. Complex II, shown in dark green rectangle, is the succinate dehydrogenase, Complex III (shown in purple oval) is the Q-cytochrome c oxidoreductase, complex IV (grey rectangle) is the cytochrome c oxidase and complex V (red bulb-like shape) is ATP synthase. Electrons are taken from complex 1 and transferred along to the complexes until it reaches ATP synthase. A small electron carrier, shown in blue circle on the image, known as cytochrome C, is also required for electron transfer (Adapted from Science Facts, 2020)

Walczak et al.,2018 discovered decreased expression of the mitochondrial complexes and decrease in mitochondrial membrane potential in mitochondria of ALS patients (Cunha-Oliveira et al., 2020). Therefore, suggesting that patients with sporadic ALS have mitochondrial impairment and may have potential to cause neurodegeneration. According to Smith, Shaw, and De Vos (2017), aggregated mitochondria with structural defects had a bloated and irregular shape including neuronal cells experiencing neurofilament accumulations and axonal swelling. Aggregation of mitochondria can impair respiration, thus

diminishing ATP synthesis and triggering an apoptotic response to result in neuronal cell death.

3. APOPTOSIS

Apoptosis is a controlled death process (Schoser, Wehling and Blottner, 2001) which involves changes in neuronal cell body size and reduced axonal terminal fields. Since damaged neurons are unable to execute their basic processes, apoptosis appears to be involved in the pathophysiology of ALS.

Bcl-2 proteins are able to regulate apoptosis however a cascade of interactions can activate caspase proteases leading to morphological changes of apoptosis (Cory and Adams, 2002). In mice with the G93A mutation in SOD1, anti-apoptotic Bcl-2 expression delayed the development of motor neuron degeneration (Mu et al., 1996). Therefore, by increasing Bcl-2 expression and delaying neuronal loss in patients with ALS could ease symptoms such as paralysis of muscle movement. In addition, Rowland and Shneider 2001 suggest inhibiting programmed cell death could aid the later stages of neuronal degeneration found in ALS. However, it is important to maintain balance between pro- and anti-apoptotic members of the Bcl-2 family for cell viability.

Shinoe et al. 2001 hypothesised that apoptosis activator protein, BH3-only peptide harakiri (Hrk), is expressed largely in the central nervous system (CNS) of patients with ALS. The balance of pro- and anti-apoptotic proteins makes it difficult to directly link Hrk expression to the death of neuronal cells, however a role in the aetiology of ALS might be established. In human ALS spinal neurons, heterodimerization interactions between Hrk and Bcl-2 indicate an overexpression of pro-apoptotic Hrk (Shinoe et al. 2001). To further understand the protein's intracellular distribution and the involvement of Hrk in the pathophysiology of ALS, more research in this area is necessary.

4. NEED FOR BIOMARKERS

Due to complex molecular pathogenesis, neurological illnesses are difficult to diagnose in the early stages. Only clinical examinations, comprising of electromyograms, nerve conduction studies, MRIs, blood and urine samples, lumbar punctures, and muscle biopsies, are currently available as tests for ALS, thus causing delayed diagnosis during disease progression. Although there is no cure for ALS, available treatments can improve quality of life. Riluzole, an oral medicine, can prolong life by three to six months (Zoccolella, 2009), however side effects include changes in liver function and dizziness. Edaravone is a drug that can lessen the decline in everyday functioning, but adverse effects such as bruising, and shortness of breath may be experienced. Relyvrio is a recently licenced drug that can aid in performing daily duties however side effects include nausea and upper respiratory infections. Currently, patients are offered therapies that focus on maintaining daily functions such as: mobility, strength, speech, and dietary needs (NHS Choices, 2021).

Treatment aims to improve quality of life however it is impossible to reverse the damage caused by the disease. Therefore, it is essential to have a conclusive ALS test that could pre-clinically diagnose people during the disease's early asymptomatic stages for better results for treatment options. Witzel, Mayer, and Oeckl 2022 state that up to 10% of ALS diagnoses are false positive, while up to 44% may be false negative, thus resulting in common misdiagnosis due to the lack of an accurate and definite test for ALS. Biomarkers are important for illness diagnosis because they can be reliably measured and evaluated as a proxy signal for the pathophysiological process, reducing the incidence of misdiagnoses. Although ALS is a rare disease, it is the most frequent kind of motor neuron disease (Witzel, Mayer and Oeckl, 2022). Several motor neuron diseases overlap with clinical symptoms, thus forming a challenge to correctly define and classify subpopulations of motor neuron disease patients. Finding a single biomarker with a high diagnostic predictive value would be difficult, since ALS is considered to have overlapped clinical symptoms (Longinetti et al., 2017).

The progression and severity of chronic neurological diseases can be monitored using biomarkers. Neurological biomarkers fall into four primary categories: blood, immunohistochemical, neuroimaging, and electrophysiological (Reddy and Abeygunaratne, 2022). In this research project, the aim is to determine early occurrences that might contribute to the pathogenesis of ALS. An accurate pre-clinical diagnosing biomarker(s) could be

identified that targets pathogenic pathways which leads to ALS phenotype. Therefore, it is crucial to gain a better understanding of the causes and pathophysiology of ALS.

5. BIOMARKERS

Biomarkers, or biological markers are molecules found in body fluids, such as blood, or from tissue samples, and are classed in 7 categories as illustrated in Figure 5. Quantitative analysis of these molecules can reveal biological characteristics enabling clinicians to determinate individuals at risk of developing a certain disease or patient's reaction to treatment (Lundblad, 2016). Neuronal biomarker discovery is empowered and driven by medical neuroscience research, however, the establishment of biomarkers for neurological diseases has been hampered by the complexity of the nervous system and limited access to tissues by the blood-brain barrier (Davis et al, 2008).

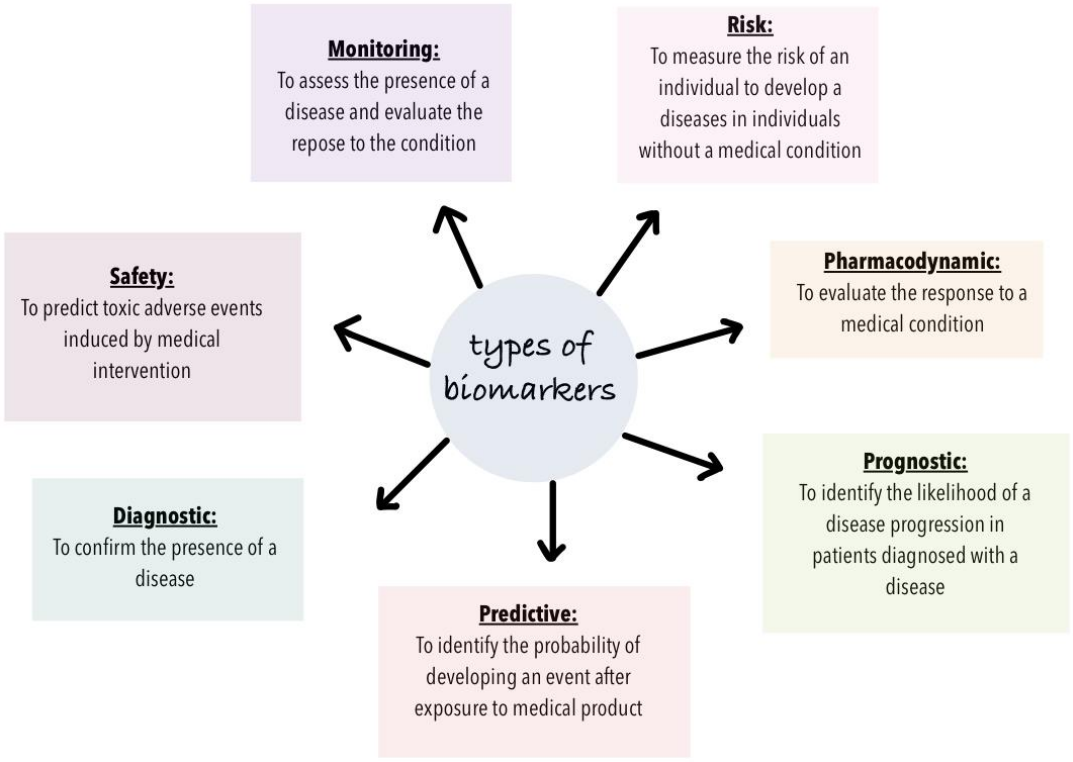

Figure 5: Different types of Biomarkers. A spider diagram that illustrates the different kinds of biomarkers and their clinical applications. (Adapted from Manzanares et al., 2021)

The ability to measuring risk factors for neurological disease enables scientists to analyse direct measurements for exposure to risk factors or biological alternations to decrease misunderstanding towards disease pathology (Mayeux., 2004). This improves the sensitivity, ability to detect disease present in patients, and specificity, ability to distinguish patients in whom disease is absent, thus allowing formation of accurate conclusions for better treatment options. Additionally, biomarkers can also be used to evaluate the effectiveness of a particular treatment, leading to more personalized treatment plans.

With the development of new technologies, it is likely that the use of biomarkers will continue to grow in the future.

The term, biosignature refers to the amalgamation of various biomarkers to provide a more precise outcome (Davis et al., 2008). Enhanced understanding of disease pathology could be obtained through a combination of tradition and novel biomarkers targeting diagnostic, predictive and pharmacological ability. A successful example was the combination of genomes and imaging tools that led an increased diagnostic sensitivity and specificity than either independent variable alone (Davis et al., 2008). However, screening for early diagnosis of a disease without a plan of therapeutic action is futile because the possibility of successful treatment is unavailable.

A combination of clinical data, risk factor analysis and biomarker specificity or sensitivity will yield successful results, however identification of a distinctive biomarker is challenging since neurological diseases have the ability to mimic one another. Complications in the discovery of biomarkers arise due to necessities of an ideal biomarker which, according to Reddy and Abeygunaratne, 2022, should be easily quantifiable, reproducible, inexpensive, and constant under environmental exposure. Registration of trials with the Medicines and Healthcare products Regulatory Agency (MHRA) for evaluating reliability, accuracy, and robustness of biomarkers can cause issues in approval despite being a crucial step in biomarker discovery (Antoniou et al., 2019).

6. Current Biomarkers for ALS

6.1 $p75^{ECD}$

Neurotrophins are proteins essential for developing, maintaining, and regulating function of the nervous system in humans. There are two classes of receptors that are activated by neurotrophins: Tyrosine kinases (Trk) and p75 (Huang and Reichardt, 2001). p75 exhibits a death domain that can mediate apoptosis upon ligand binding. (Rabizadeh and Bredesen., 2003). Neurotrophin receptor, p75, is mainly expressed in early years of human development and after motor neuron injury in adults which could mean a potential biomarker for ALS.

An increased level of p75 expression was observed in lumbar motor neurons of mice with ALS and cervical spinal cord of human patients with ALS (Lowry et al., 2001). Copray et al., 2003 suggested a proapoptotic role of p75 due to finding interactions of p75 activating apoptosis protein caspase 3. Since both studies display similar findings, p75 could initiate a death-signalling cascade contributing to the pathogenesis of motor neuron degeneration in ALS.

Research conducted by Shepheard et al., 2017 led to the identification of p75ECD, a specific fragment of protein p75, as disease progression and prognostic biomarkers in ALS. The prognostic value of baseline $p75^{ECD}$ was explored by survival analysis, a statistical method for analysing the expected duration of time until death or failure occurs. The study results confirmed higher concentrations of $p75^{ECD}$ was found in patients with ALS compared to controls and reproducibility of assay was high. However, it is uncertain if the study considered different forms of ALS: familial or sporadic. Additionally, Shi et al., 2021 conducted a meta-analysis, supporting the use of urinary $p75^{ECD}$ as a diagnostic biomarker and determining its ability as a progression indicator. Further research into understanding why levels of $p75^{ECD}$ increase in urine as disease progresses would be beneficial. It is unclear if this can be used as a pre-symptomatic test for determining ALS in patients and perhaps the link between increased levels of $p75^{ECD}$ could lead to increased beneficial research.

6.2 CSF Neurofilament Proteins

The cerebrospinal fluid (CSF) is an ideal target for biomarker discovery since there is contact with motor neurons in the brain and spinal cord. The changes in the biochemistry of the CSF

could be indicative of neuronal injury and neurodegeneration leading to accurate results during pre-clinical tests. (Brettschneider et al., 2006). According to the review by Lee and Cleveland 1996, structural stability and axonal polarization of cells is ensured through vital neuron-specific cytoskeletal proteins called Neurofilament light chain (NF-L) and heavy chain (NF-H). Both neurofilaments have elevated levels in the cerebrospinal fluid and blood in patients with ALS, which may be useful biomarkers for ALS (Rosengren et al., 2002). However, the mechanisms behind the elevated neurofilaments were not explored.

Phosphorylation is a post-translational modification regulating neurofilament transport, however, accumulation can lead to neuronal degeneration (Dale and Garcia, 2012). A study conducted by Rossi et al. 2018, measured NF-L and phosphorylated NF-H (pNF-H) concentrations in the cerebrospinal fluid of ALS patients and found a significant increase compared to control. pNF-H appeared to be slightly more efficient than NF-L, which could be due to the post-translation modification allowing resistance against protease degeneration thus longer survival rates. NF-L may have prognostic relevance because increased centration in CSF levels seem to correlate with shorter survival rates. NF-L is relative to axonal loss which may reflect later stages in neuronal degeneration, further enforcing prognostic value (Alirezaei et al., 2019). Sensitivity and specificity were observed in CSF neurofilament, between ALS patients and controls, and results conclude that both neurofilaments show a relatively good performance as diagnostic biomarkers for ALS. This post-translational modification is a suitable target for biomarker discovery since early stages of disease pathology could be assessed leading to a pre-symptomatic test for ALS.

Verde et al. 2019, conducted a study to determine the diagnostic and prognostic performance of serum neurofilament light chain in ALS. They opted for a longitudinal study consisting of patients that had neurodegenerative disease. Serum NF-L levels were measured using ultrasensitive single molecule array (Simoa) technology. It allows detection of proteins and nucleic acids at lowest possible levels through isolation of individual antigen-antibody complexes on paramagnetic beads. Fluorescence emitted by the analyte allows determination if each individual bed is bound to the target analyte (Quanterix, n.d.). The results displayed higher level of serum NF-L in patients with ALS compared to all other categories, with 85% sensitivity and 81.8% specificity. Meaning, serum NF-L has the ability to both detect the disease in patients and ability to determine if the disease is absent within the patient. Serum

NF-L correlated positively with disease progression rate among patients with ALS which further validates hypothesis of NF-L as prognostic biomarker from Rossi et al 2018.

CSF extraction for pNF-H involves a challenging procedure for many patients and continuous lumbar puncture is an unrealistic scope outside clinical research (Staats et al., 2022). Perhaps in the future, there could be a possibility to move away from lumbar punctures since serum-based NF-L appears to be a good prognostic candidate. It is unclear if pre-symptomatic stages can be targeted through neurofilaments, but pNF-H was found to be more efficient (Rossi et al, 2018), and further studies of pNF-H accumulation could be targeted as an indication for early ALS onset.

7. EMERGING BIOMARKERS

7.1 Serum miRNA

MicroRNA (miRNA) are single-stranded, non-coding RNA molecules comprised of 21 to 23 nucleotides. Involved in RNA silencing and post-transcriptional regulation of gene expression via binding of messenger RNA (mRNA) in the cytoplasm (Cannell, Kong and Bushell, 2008). Currently, diagnosis of ALS relies upon a patient being referred to a neurologist for evaluation (Cellura et al., 2012). miRNAs are being applied more frequently in the field of neurological disorders for diagnostic and prognostic reasons (Ma and Weinberg, 2008).

Gene expression is prone to post-transcription, through processes such as phosphorylation, and are regulated by miRNAs through binding to complementary sections of the targeted mRNA. According to Daniels et al., 2014 there are measurable quantities of miRNAs in blood, meaning they can be clearly acquired through non-invasive procedures. Study conducted by Botta-Orfila et al., 2014 found blood-derived miRNAs are highly resistant to RNase degeneration, a variety of storage temperatures, low/high pH conditions, and repeated freeze-thaw cycles making them potential biomarkers. This is also in accordance with targets set by Reddy and Abeygunaratne, 2022 for an ideal biomarker. miR-143-3p expression was elevated in the serum of sporadic ALS (sALS) patients (Waller et al., 2017), which may aid in our understanding the disease pathophysiology and the development of biomarker detection. Although the study did not analyse familial ALS subgroups, ALS-mimicking diseases with various neuromuscular conditions were compared. Further research is needed to understand the function of miRNAs and how they might be used as biomarkers in ALS.

miRNA signatures from the serum of ALS patients were identified using initial miRNA profiling, and results found 3 differently expressed miRNAs of which miR-206 and miR-143-3p expression was increased but miR-374b-5p expression decreased (Waller et al., 2017). Waller et al 2017, suggest that decreased miR-374b-5p could be a compensatory effect to restore muscle loss in later stages of ALS through increased myoblast differentiation. The increased expression of miR-143-3p reflects the progress of muscle degeneration thus exhibiting prognostic qualities. Opposing expression profiles displayed could lead to a miRNA signature comprising of both mir-143-3p and miR-374b-5p to assess treatment efficacy through measuring changes in muscle damage. This would not be used as a pre-

symptomatic predictor for ALS as muscle damage occurs in the final stages of disease progression.

Upregulation of miR-206 in SOD1-G93A mice was found in a study by Toivonen et al., 2014, consistent with results from Waller et al., 2017. miR-206 has a high abundance in skeletal muscle tissue but is found in blood as a waste product released from muscle fibres after rhabdomyolysis, muscle tissue death. mir-206 has been identified as a blood-based biomarker for muscle-related diseases (Coenen-Stass, Wood and Roberts, 2017) but could be useful in distinguishing from ALS-mimicking conditions in a biosignature.

7.2 Glutamate

Glutamate is an amino acid that acts as an excitatory neurotransmitter in the central nervous system. Usually, it is involved in cognitive functions such as memory and learning as well as formation of synapses during brain development (Blasco et al., 2014). 40% – 75% of patients with sporadic ALS are estimated to have increased levels of extracellular glutamate due to defects in glutamate transport (Spreux-Varoquaux et al., 2002), however the effect on familial cases is unknown.

Ecotoxicity can occur due to overstimulation of glutamate receptors to increase flow of calcium ions into the neuron, known as cytosolic Ca^{2+}. This can activate enzymes, such as phospholipases that damage cell membrane, cytoskeleton, mitochondria, and DNA eventually leading to apoptosis (King et al., 2016). Modifications in glutamate receptors might be linked to the pathophysiology of ALS as modification of the receptor subunit occurs in motor neurons of people with ALS (Kawahara et al., 2004). Limited functional transport of glutamate and reduced EAAT2 immunoreactivity, as described in post-mortem tissue from ALS patients (Rothstein et al., 1995), can lead to neuronal death. Loss of EAAT2 has been shown to contribute to disease progression in SOD1 transgenic G93A mice models of ALS (Guo, 2003). Due to reduced glutamate uptake from the cytoplasm, there is an increase in extracellular levels of glutamate correlating to reduced EAAT2 expression in pre-symptomatic stages. However, is undetected during the later stages of disease progression in transgenic models (Howland et al., 2002). Meaning, assessing transport of glutamate across the synapse could provide information on EAAT2 expression leading to apoptosis thus a potential to predict onset of ALS in the early stages. However, assessing levels of glutamate

in CSF or blood may not reflect levels in the synapse and further research in finding methods to assess glutamate levels in synapses should be investigated.

Evaluation for the potential for glutamate to act as either a diagnosis or disease progression marker had led some studies to find that glutamate-provoked ecotoxicity in the pathophysiology of ALS is due to increased glutamate in the CSF of ALS patients (Plaitakis and Caroscio 1987, Rothstein et al., 1990). However, conflicting metabolomic studies show decreased glutamate concentration (Wuolikainen et al., 2011) or increase (Blasco et al., 2013) in CSF. The conflict may arise due to different analysis methods, since Wuilikainen et al 2011, used gas chromatography coupled to mass spectrometry (GC/TOFMS) and multivariate statistical modelling. Whereas Blasco et al 2013, used liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS) to assess the analyte. The effect of glutamate levels in different tissues at different stages of disease progression should be identified to understand potential of glutamate to act as a progressive biomarker.

8. DIAGNOSTIC TECHNIQUES

8.1 T-SICI

In an attempt to allow early detection of upper motor neuron dysfunction, part of ALS pathology, Menon et al 2015 assessed the sensitivity and specificity of a threshold tracking transcranial magnetic stimulation (TMS) technique.

TMS is a non-invasive procedure in which electrodes are placed on the scalp to stimulate nerve cells within the brain, and therefore assess the function of upper motor neuronal signals originating from the cerebral cortex (Floyd et al., 2009). Later, this developed into a serial threshold tracking SICI (T-SICI) system which measures the reduction in the response to a strong TMS pulse, which is greater than necessary to reach threshold, and a smaller TMS pulse that is weaker than the threshold of firing an action potential. Measurements of relative amplitude reduction of motor evoked potential (MEP) in peripheral nerves and muscles after cortical or spinal stimulation, measures the integrity of motor pathways (Fisher et al., 2002). Due to the high level of diagnostic effectiveness, it has been advocated as a potential biomarker for ALS, and other academics (Tankisi et al., 2021a) have investigated this strategy and agreed that it might be used as a potential ALS biomarker.

In the experiment by Tankisi et al 2021a, the T-SICI measurements were able to distinguish that 59.6% of the patients had motor neuron disease, with the remaining patients obtaining various diagnoses. The criteria for ALS disease were met by 75.8% of patients with underlying motor neuron disease (Tankisi et al., 2021a). The remaining patients were either diagnosed based on a clinical follow-up or had merely lower motor neuron involvement that was confirmed by needle electromyography (EMG). Although the technique is reliant on pre-identification of motor neuron disease, it was able to reliably distinguish ALS from non-ALS disorders. It would be beneficial to conduct a larger replication of this study as it could be used as an objective biomarker for detecting upper motor neuron dysfunction in earlier stages of ALS.

8.2 Imaging tools

Imaging maintains a unique position in the contemporary practise of medicine and also offers tremendous potential for the discovery of new biomarkers. The two most used structural biomarkers for the nervous system are computed tomography (CT) scans and magnetic

resonance imaging (MRI) (Varghese et al., 2013). The primary objective of traditional MRI imaging of the brain and spinal cord is to rule out probable ALS mimics (Agosta et al., 2013).

8.2.1 Magnetic Resonance Spectroscopy

Magnetic resonance spectroscopy (MRS) is an analytical method that enables identification and quantification of regional brain metabolites, such as N-acetyl aspartate (NAA) or glutamate. NAA is synthesized by neurons, and it is one of the most abundant brain metabolites. However, the purpose of secreting this molecule requires further understanding (Moffett et al., 2013). Due to its digital nature, biological model characteristics like grey matter iron deposits as well as picture features like texture or morphological descriptors can all be quantitatively measured (Mazón et al., 2018).

Detection of upper motor neuron loss is possible through MRS technique. Reductions in NAA concentrations have been demonstrated through multiple MRS studies on patients with ALS. Mitsumoto et al., 2007 concluded that measurement of primary motor cortex, resulted in reduced NAA concentration through MRS which correlates a significant difference between ALS and controls. These results increase the possibility that MRS can provide an insight into the pathogenesis of motor neuron diseases. The results also are similar to Pioro et al 1994 study which found a reduction in NAA concentration through use of proton magnetic resonance spectroscopic imaging in patients with motor neuron disease. To further enforce the idea of utilizing MRS in detecting early disease changes in ALS, studies have suggested a correlation between disease severity and reduction of NAA concentration (Ellis et al., 1998). Increased sensitivity towards detecting upper motor neuron degeneration (Kaufmann et al., 2004) was identified through a combination of MRS and diffusion tensor imaging (DTI). Therefore, increasing the potential of diagnosing ALS through a combination of other digital techniques.

This suggests that a promising metabolite that accurately reflects upper motor neuron involvement, is motor cortex NAA. Detecting concentration changes of this metabolite could be beneficial for detecting early pre-symptomatic stages of ALS. Technological advancements that can offer increased resolution for whole-brain imaging and utilizing artificial intelligence to process the information might offer further insights and possibilities for this technique.

8.2.2 Structural Magnetic Resonance Imaging

Structural magnetic resonance imaging is a non-invasive technique used to examine both the anatomy and pathology of the brain. The ability to distinguish between grey and white matter volume allows measurements using voxel-based morphometry (VBM) and surface-based morphometry (SBM) (Verstraete and Foerster, 2015). A volume pixel is called a voxel, and it is the 3D version of a 2D pixel.

VBM is an objective method, which requires inferring data presented to enable the assessment of grey and white matter and the comparison of each voxel in patients. It also evaluates the density of grey and white matter (Mazón et al., 2018). VBM studies have detected abnormalities in the primary motor cortex of ALS patients (Kassubek et al., 2005, Chang et al., 2005), however there is inconsistency amongst the results (Chiò et al., 2014), meaning the data presented is less reliable. A consistent cortical thinning of the primary motor cortex in patients with ALS has been identified through SBM research (Al-Chalabi et al., 2016). Assessing changes in cortical thickness allows evaluation of upper motor neuron dysfunction indicating that morphometric techniques could be utilised as neuroimaging biomarkers for ALS. Since SBM results show inconsistencies, VBM is less trustworthy and sensitive to atrophy changes in the cortical motor areas than SBM.

Shen et al. 2016, performed a voxel-wide meta-analysis using 29 VBM studies and discovered that even in the absence of symptomatic cognitive deficits, presence of atrophy, primarily in the primary motor cortex confirm structural brain changes in ALS. Alterations in the cortical thickness of the motor cortex are also a crucial indicator of ALS pathology (Verstraete et al., 2012b). Cervo et al. 2015, found that combining MRI and MRS increases diagnostic accuracy in ALS. Perhaps a combination of VBM studies and MRS could increase accuracy of findings to provide more reliable data and could enhance our understanding of abnormalities in the primary motor cortex of patients with ALS. It might not be possible to use these techniques during the early pre-symptomatic stages of ALS, since changes in brain structure would arise from degeneration of neurons that is seen during the secondary stages of disease progression.

8.2.3 Functional Magnetic Resonance Imaging

Functional magnetic resonance imaging is a non-invasive technique to measure brain activity by detecting changes in blood flow. The technique works due to the idea that neuronal activation and blood flow are coupled, therefore an active area of the brain requires an increased blood flow. This gives rise to the blood oxygenation level-dependent (BOLD) approach, which measures the imbalance in cerebral blood flow and deoxyhaemoglobin concentration to analyse brain activity (Agosta et al., 2010).

To examine the functional organisation of the brain, resting state functional MRI detects spontaneous low-frequency fluctuations in the BOLD signal. Alterations in premotor brain cortex, which lies in front of the motor cortex, and functional connectivity in ALS patients have been observed. Studies comparing ALS patients to controls, have shown lower functional connectivity within the sensory motor networks and anomalies in networks associated to cognition and behaviour (Tedeschi et al., 2012, Fekete et al., 2013, Zhou et al., 2013). However, some researchers have suggested that somatosensory and other motor regions, as well as network coherence, are increasing in their activity (Fekete et al., 2013, Luo et al, 2012).

Greater functional connection acts as a form of structural damage compensation since disease pathology is increasing. Another reason could be signs of early disease characteristics decreased local inhibitory function (Turner and Keirnan, 2012), and focusing on the early disease pathology could indicate a potential pre-symptomatic biomarker for ALS.

8.2.4 Diffusion Tensor Imaging

Diffusion tensor imaging (DTI) is a method for characterising microstructural changes or observing changes in neuropathology with treatment. DTI signals relate to the random movement of water molecules within an MR voxel. Molecular mobility facilitates diffusion, although might be restricted in some directions, similar to white matter anisotropy or dependence on direction, as diffusion is a three-dimensional process (Le Bihan et al., 2001). The three primary DTI measurements as follows: mean diffusivity, axial and radial diffusivity, and fractional anisotropy (FA). White matter fibre anisotropy, myelin integrity,

density, and fibre parallelism are all key indicators of FA, a scalar assessment of diffusion movement. Therefore, FA lacks direction and only comprises of magnitude. According to Mazón et al. (2018), mean diffusivity, also known as apparent diffusion coefficient (ADC), is the average movement of water molecules within the voxel.

In DTI studies, ALS patients regularly have decreased FA and elevated ADC of the corticospinal tract (Rosskopf et al., 2015). Most alterations throughout the corticospinal tract occur in the posterior limb of the internal capsule, containing corticospinal and sensory fibres. Thus, meaning that changes in this area affects movement and co-ordination which are symptoms seen in later stages of ALS. FA changes in corticospinal tract as shown in a meta-analysis by Foerester et al 2012, were consistent. However, ability to distinguish ALS patients from healthy controls was disappointing. A decrease in FA alterations in corticospinal tract relates to the severity of the disease and the rate at which the disease progresses indicate upper motor neuron degeneration (Mazón et al., 2018).

Since DTI studies revealed reduced FA in the white matter below the right primary cortex and the corticospinal tract (van der Graaf et al., 2011), DTI may be an early indicator for ALS and clinically silent upper motor neuron involvement (Sach et al., 2004). By identifying abnormalities to the white matter tract in the motor and extra-motor areas, DTI may enable ALS phenotyping.

DISCUSSION

ALS is a neurodegenerative disease, resulting from deterioration of upper and lower motor neurons in both the brain and spinal cord. This leads to a progressive loss in muscle coordination, promoting muscular atrophy and progressive bulbar palsy. The final stage of ALS occurs when the body becomes completely paralyzed. Individuals at this stage experience difficulties in breathing, speaking, or swallowing and might require both a ventilator and a feeding tube. Currently, ALS is diagnosed through multiple clinical examinations including electromyograms, nerve conduction studies, MRIs or blood samples taken from patients. However, this can only be conducted when symptoms are present, thus preventing an early diagnosis. Due to the nature of disease progression, when the patient begins experiencing symptoms, too much damage has occurred in order to reverse or terminated the disease.

By considering early pathogenesis of ALS and targeting those sites, a biomarker could be distinguished that provides accurate pre-clinical diagnosis for better treatment options. Many studies have pursued to identify biomarkers that target ALS pathology and allow for earlier diagnosis (Vu and Bowser, 2016, Shepheard et al., 2017). However, since neurological conditions mimic symptoms, it is difficult to distinguish ALS from other motor neuron diseases. Therefore, a biomarker must have high specificity and sensitivity to provide accurate results in determining individuals with ALS.

pNF-H and NF-L found in the CSF of ALS patients have emerged as putative diagnostic biomarkers (Rosengren et al., 2002). They seem to be most promising biofluid based biomarkers for ALS as proteins arise parallel to symptoms (Vu and Bowser, 2016). pNF-H has shown to be more efficient than NF-L (Rossi et al. 2018), perhaps due to the post-translational modification increasing resistance and survival rate. However, NF-L can be extracted through less invasive procedures than pNF-H. Therefore, future investigations using a combination of other biomarkers could lead to a pre-symptomatic and prognostic biomarker discovery.

A recently discovered potential biomarker within serum, miRNAs, has been recently discovered, specifically miR-206, 143-3p and 374b-5p. Waller et al. 2017 suggest miRNAs are useful as prognostic biomarkers as higher concentrations correlate to an increase in disease progression. However, miRNA-206 is a biomarker for muscle degeneration (Coenen-Stass, Wood, and Roberts, 2017) and could potentially distinguish ALS from other neurological

conditions leading to muscle wastage, when combined in a biosignature. Although these findings are beneficial, they are unable to diagnose ALS before symptoms arise.

A combination of biological biomarkers (biosignature) and diagnostic techniques would provide an increased sensitivity and specificity for ALS while reflecting the rate of decline throughout the disease progression. Verde et al. 2019 found that NF-L resulted in high specificity and sensitivity for ALS and since levels can be assessed in parallel to symptoms, it can be a reliable biomarker for early diagnosis and prognosis. MRS can detect concentrations of NAA which can indicate upper motor neuron degeneration that occurs in ALS pathology (Moffett et al., 2013). However, an updated MRS technology with higher resolution to form whole-brain imaging would increase the sensitivity of detecting metabolites in the brain.

Overall, a combination of NF-L and MRS would yield successful pre-symptomatic assessment and accurate reflection of disease progression for ALS. Future studies targeting artificial intelligence (AI) to discover biomarkers or treatments for ALS would be beneficial. AI has been increasingly helpful in the medical field, whether diagnosing patients (Yao et al., 2021), drug discovery and development (Matin, Taghian and Chitsaz, 2022) or ability to remotely treat acute illnesses. Till date, there is no published research which involves AI and ALS, however, an AI-driven pharma-technology company, Insilico Medicine have initiated personalised research and drug discovery for ALS. In 2016, the company utilized AI to identify novel targets for new ALS drugs. However, the efficacy of the proposed targets was inconclusive (als.ai, n.d.). Further investigations using this technology could provide successful results and may be able to identify pre-symptomatic biomarkers to detect ALS.

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